THE ANALYSIS OF POLARIZED LIGHT IN THE EYE OF DAPHNIA[1]

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The operation of any polarization detector involves: (1) separation of incident light into two vectors perpendicular to each other and to the direction of propagation of the light beam, (2) suppression of one vector, (3) intensity estimation of the remaining vector. In physical instruments polarization analysis depends on rotation of the analyzer around the direction of propagation of the light beam. In the biological systems found in arthropods, polarization analysis depends on the possession of a radial array of analyzers, whether this is the rhabdomere-retinula cell complex or the many different corneal and lens surfaces of the compound eye of arthropods. Such an array of analyzers permits simultaneous comparison of polarized intensities present at all azimuths about the direction of propagation of the light beam. These comparisons may be between different ommatidia, with the receptor system of each ommatidium acting as a unit, or between parts of the receptor system of a single ommatidium.

Three models have been proposed to account for the orientation of animals to polarized light, of which two are intra-ocular, the third extra-ocular. The three models are: (1) the Brewster-Fresnel model in which one or more refractions and reflections at corneal or lens surfaces serve to alter preferentially the intensity of light polarized parallel to the plane of incidence; (2) dichroic filters (the rhabdomeres) with the fast axes tangential to the radii of the array of filters; (3) the reflected brightness pattern in which the intensity of light reflected or scattered from the environment is greater perpendicular to the light polarization plane.

The Brewster-Fresnel refraction model relying upon a single refraction was proposed by Stephens, Fingerman and Brown (1953) for the *Drosophila* eye. The Brewster-Fresnel reflection model relying upon internal reflection from a lenticular surface was proposed by Baylor and Smith (1953) for *Daphnia*. The presence of dichroic filters in the eye of the bee was suggested by Autrum (see von Frisch, 1950) and has been supported by Stockhammer (1956, 1959). The environmental reflection pattern as an orientation stimulus was suggested by Baylor and Smith (1958).

Values of the theoretical light intensities calculated from the Fresnel equations are compared with actual measurements through two surfaces of the daphnid cone lens. The data presented here support the first of these intra-ocular models for the eye of *Daphnia pulex* (de Geer).

MATERIALS AND METHODS

The measurements were made on the lenses of freshly killed daphnids mounted in water under a coverslip on a microscope slide and examined at 500 × under a

Leitz Ortholux microscope with a trinocular head. A rotatable type N Polaroid filter was interposed in the light beam beneath the microscope condenser. A 10 × ocular fitted with a field stop diaphragm replaced the camera on the trinocular head and was coupled to a photomultiplier photometer (Photovolt model No. 501M) for measuring light intensities. The field stop diaphragm placed at the image plane of the ocular restricted the field of view of the photomultiplier to a circle 10 μ in diameter. The center of the circle was coincident with the intersection of crosshairs in one of the viewing oculars to permit location of the desired area.

The change in light transmission through the carapace adjacent to the eye was measured as the Polaroid was rotated through 90° from a position parallel to the preferred transmission plane of the microscope to one perpendicular to that plane. Individual lenses were aligned with the preferred transmission plane of the microscope and the change in transmission of refracted light was again measured with the Polaroid in two positions. These measurements were repeated with the long axis of the lens rotated 90°.

RESULTS AND DISCUSSION

The measurements from 24 cone lenses from 6 different eyes are summarized in Table I. Columns 2 and 5 contain data based on the measured intensities of light transmitted through the carapace alone. These percentages are calculated by dividing the reading of the photometer with the Polaroid in the east-west position by the reading with the Polaroid in the north-south position (NS/EW × 100), where EW is the preferred transmission plane of the optical system of the microscope. This always gives a calculated value of less than 100. A comparison of columns 2 and 5 shows the difference due to the carapace alone, because the carapace has been turned through ninety degrees. The differences measured in this manner are small, and are probably random, indicating the carapace is not an effective polarization analyzer. A comparison of columns 1 and 2, and columns 5 and 6 shows not only the difference between the effectiveness of the lens and the effectiveness of the carapace but also that the preferred transmission plane of the hemispherical lens is parallel to the long axis of the cone. In columns 2 through 4, with the lens oriented NS, the average transmission of the background, 77.9%, is exceeded by that of the lens, 81.3%, a difference of 3.4%. This is because the lens transmits a greater proportion of the light when the Polaroid is in the NS position, that is, parallel to the lens axis. In columns 5 through 7 the reverse is true: the lens faces EW, its preferred plane is EW and therefore the fraction NS/EW becomes smaller when the Polaroid is turned from EW to NS, that is, perpendicular to the lens axis. The mean observed change is from 77.3% to 74.2%, a difference of 3.1%. Columns 4 and 7, taken by themselves, measure the change in intensity corrected for the lens system of the microscope, with the difference in column 4 being positive, that in column 7 negative. Of the 48 readings, four differ in a direction opposite to the expected direction. The mean difference of all the measurements, correcting for the difference in sign, is 3.25% and lies between 2.0% and 4.5% with a probability greater than .99. This difference is consistent with the calculated values for the Brewster-Fresnel reflection model. Reference to Figure 1 shows that measurements have been made on light diffracted both at point B and at point D.

TABLE I

Measured values of light intensity

	Lens NS			Lens EW		
	% transmission of background	% transmission of lens	Difference	% transmission of background	% transmission of lens	Difference
1	81.00	83.75	2.75	76.50	71.59	−4.91
2	76.50	85.00	8.50	77.00	75.00	−2.00
3	77.00	82.24	5.24	77.00	73.74	−3.26
4	77.00	80.62	3.62	76.50	72.83	−3.67
5	78.00	96.67	18.67	77.00	75.38	−1.62
6	77.70	78.57	0.87	77.50	73.38	−4.12
7	80.00	82.02	2.02	77.00	73.38	−3.62
8	76.00	82.43	6.43	77.50	74.52	−2.98
9	76.00	83.78	7.78	78.50	72.29	−6.21
10	77.00	80.72	3.72	78.00	75.66	−2.34
11	78.00	80.49	2.49	77.50	69.87	−7.63
12	77.00	81.32	4.32	77.00	73.97	−3.03
13	79.00	79.89	0.89	78.50	75.29	−3.21
14	78.00	81.71	3.71	78.00	74.29	−3.71
15	78.00	80.00	2.00	78.00	73.54	−4.46
16	76.00	75.00	−1.00	78.00	75.34	−2.66
17	79.00	82.56	3.56	78.50	74.26	−4.24
18	79.00	78.87	−0.13	77.50	78.31	+0.81
19	77.00	79.76	2.76	75.50	74.71	−0.79
20	76.00	76.68	0.68	78.50	75.58	−2.92
21	78.00	83.33	5.33	78.50	76.37	−2.13
22	77.00	77.95	0.95	77.00	76.16	−0.84
23	82.00	77.78	4.22	77.00	72.63	−4.37
24	80.00	81.08	.108	74.00	73.85	−0.15
Mean	77.92	81.34	3.42	77.33	74.25	−3.08

Table II summarizes the calculated intensities for rays incident between 10° and 80° on the external surface of the daphnid cone lens (angle $\emptyset$ in Figure 1) and whose plane of polarization is either parallel or perpendicular to the long axis of the lens. For these calculations the index of refraction is assumed to be 1.53 and that of the blood 1.33, yielding a relative index of refraction of 1.15. Since the tangent of the angle of maximum polarization equals the relative refractive index, this angle is 49° for the external surface and 41° for the internal surface. To simplify the optical calculations and discussion, only those rays confined to a single plane are considered throughout the paper. This plane bisects the conical figure of the lens and is identical with the plane of incidence, the latter of which is defined by the incident ray and the perpendicular at the point of incidence. As is customary, the polarization plane is described as either parallel or perpendicular to the plane of incidence. For example, AB parallel means the light beam AB, which is parallel to the plane of incidence at B in Figure 1. In Table II, columns 2 through 7 contain values of reflected and refracted intensities corresponding to the labeled portions of the light paths in Figure 1. These values are referred to an intensity of 100 in the incident ray, AB.
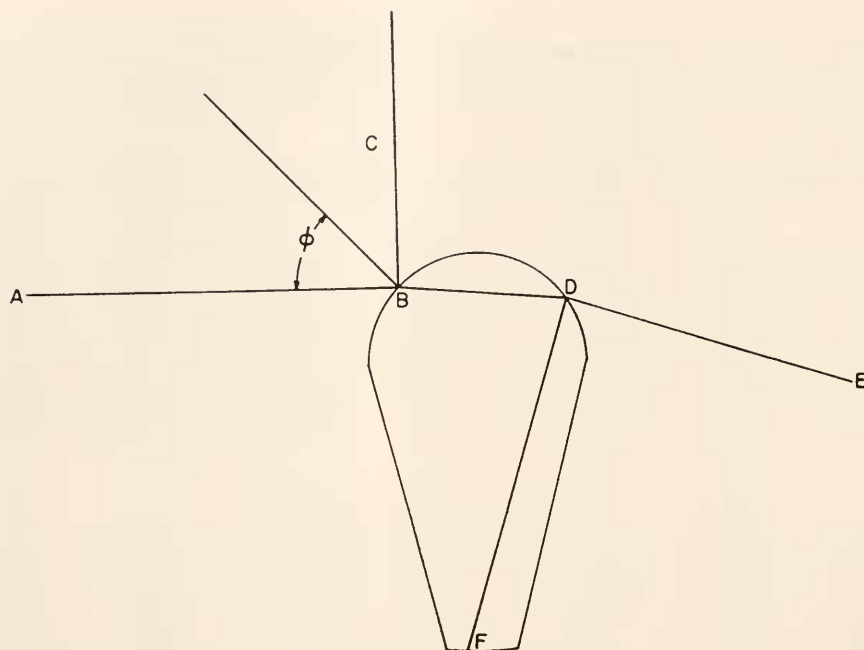
FIGURE 1. Diagram of a single cone lens showing the light path ABDF for the Brewster-Fresnel internal reflection model. Light from A is incident at B with the angle of incidence labelled $\emptyset$.

Columns 2 and 3 show the small differences which exist after the initial reflection and refraction at B; columns 4 and 5 give the somewhat greater differences occurring after two refractions at B and at D. The differences between DE parallel and DE perpendicular are given in column 9 and correspond to the measured differences of Table I. The maximum *calculated* difference at 70° is 3.28% while the mean *observed* difference is 3.0%. That these values are in such good agreement is

TABLE II

Calculated values of light intensities

1 Angle of incidence	2 $BD_\perp$	3 $BD_{\parallel}$	4 $DE_\perp$	5 $DE_{\parallel}$	6 $DF_\perp$	7 $DF_{\parallel}$	8 $DF_\perp/DF_{\parallel}$	9 $DE_\perp\text{-}/DE_{\parallel}$
10	86.16	86.21	74.24	74.32	0.45	0.40	1.125	00.08
20	85.02	85.20	72.28	72.59	0.52	0.32	1.625	00.31
30	82.90	83.34	68.72	69.46	0.66	0.21	3.143	0.71
40	79.34	80.24	62.96	64.38	0.95	0.07	13.57	1.44
50	73.36	74.89	53.82	56.09	1.51	0.15	10.03	2.27
60	63.26	65.72	40.02	43.20	2.65	0.30	8.32	3.18
70	46.26	49.68	21.40	24.68	4.73	1.77	2.67	3.28
80	20.43	24.53	4.17	06.02	6.13	4.80	1.27	1.8

The value of the incident ray, AB, is 100.

largely chance because any single measurement in Table I gives an average value for many degrees of incidence.   The fact that any difference whatsoever can be shown in Table I probably means that (1) the index of refraction is higher than the
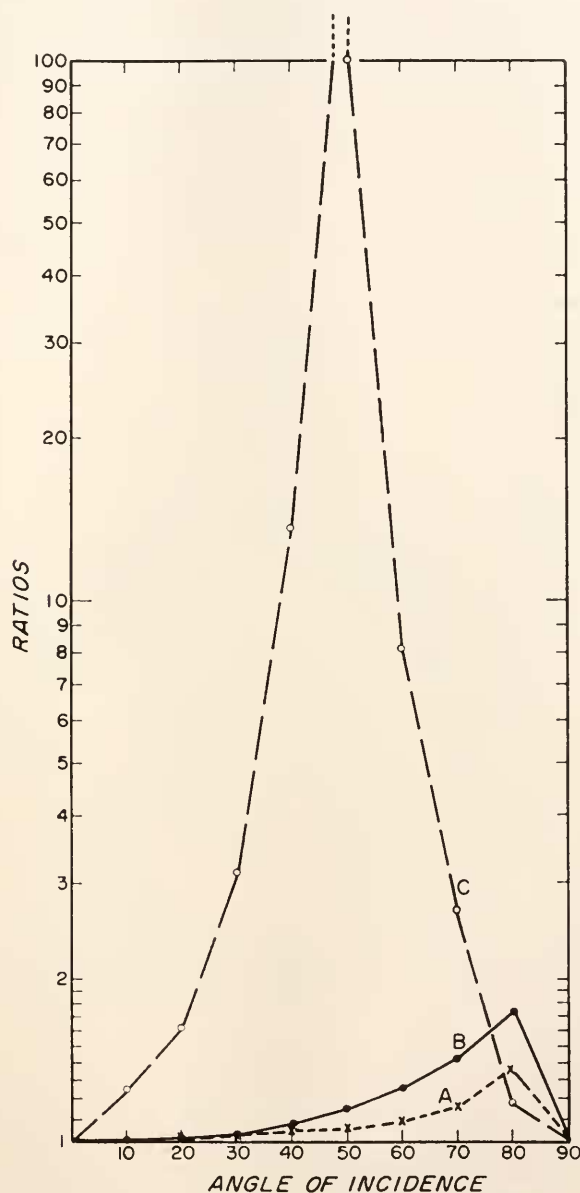

FIGURE 2.   Ratios of vector intensities resolved at corneal interfaces.   Curve A is $DE_{||}/DE_{\perp}$. Curve B is calculated from data of Stephens, Fingerman and Brown (1953) and corresponds to $BD_{||}/BD_{\perp}$ for a cornea-air interface.   Curve C is $DF_{\perp}/DF_{||}$.   The subscript $\perp$ means the ray is polarized perpendicular to the plane of incidence and the subscript || means the ray is polarized parallel to the plane of incidence.

assumed value of 1.53 and (2) the optical surfaces of the cone lens are not hemispherical and therefore the overall efficiency of analysis is higher. A more effective polarization analyzer could be realized by altering the shape of the optical surfaces so that more of the incident rays would meet the surface close to the polarizing angle. The shapes of cone lenses will be discussed later.

The effectiveness of the resolution of the refracted ray into two vectors is shown in Figure 2, where curve A is the plot of log (DE parallel/DE perpendicular). Contrasted with this is the vector resolution of the reflected ray, curve C, log (DF perpendicular/DF parallel), where the ratio of vector intensities is very high, especially near the polarizing angle. At the polarizing angle internal reflection of the parallel ray diminishes to zero and therefore the ratio approaches infinity. In this way the small differences which were measured for *Daphnia* can be associated with an effective analysis within the eye. It should be emphasized, however, that the absolute intensity of rays at the receptor in this model is low.

The effectiveness of any light polarization analyzer depends upon its ability to separate the incident light into two vectors and to present these two vectors for intensity measurement. The ratio of the intensities of the two vectors is thus a measure of the effectiveness of the polarization analyzer. The calculations in Table II permit a comparison of the effectiveness of the two different kinds of Brewster-Fresnel models which have been proposed. The first model depends upon a single refraction at the corneal surface and was proposed for the eye of *Drosophila* by Stephens, Fingerman and Brown (1953). The effectiveness of this model in resolving the incident light into two vectors depends on a high relative index of refraction characteristic of an air-cornea interface but not of a water-cornea interface. The ratios of the different vector intensities resolved by a single refraction at the cornea-air interface and by two refractions at the cornea-water interface are shown in curves A and B of Figure 2. Comparison of these two curves with curve C of the same figure shows unequivocally that refraction is not as effective as reflection for polarization analysis. It remains to be seen whether beams of light incident on the terrestrial arthropod cornea at high angles proceed by multiple reflection to the light-sensing apparatus. A sequence of such internal reflections (at less than the critical angle) would be a very effective light polarization analyzer. The observations of de Vries and Kuiper (1958) for Diptera and Waterman (1954) for *Limulus,* that ommatidia are sensitive to light incident at high angles, might be thought of as lending credence to this view. However, Waterman's (1954) work relating intensity threshold to angles of incidence raises serious doubts concerning the Brewster-Fresnel refraction model in the natural habitat because the intensity threshold for light incident near the polarizing angle is approximately 100 times greater than that of light normal to the surface. The confusion of polarized intensities with non-polarized intensities and the obscuring of any particular polarized light stimulus seem inevitable with this model unless this eye possesses an ability to distinguish 1% brightness differences.

The second Brewster-Fresnel analyzer model proposed by Baylor and Smith (1953) involves the somewhat unorthodox light path ABDF of Figure 1, which requires the light to be incident at the cornea-blood interface twice, once on entering at B and again on being reflected at D. The ratios of orthogonally polarized intensities reflected from D are plotted in curve C of Figure 2 where they give a

somewhat exaggerated impression of the effectiveness of this light polarization analyzer when the ratio of intensities goes to infinity at the polarization angle. The operation of this model may be seen in three dimensions in Figure 3. In Figure 3 the cone lenses are depicted on xyz coordinates to represent a solid figure. A ray of light parallel to the y axis and polarized parallel to the z axis is incident on the surmounting hemisphere of each of the cone lenses. The intensities resulting from subsequent refractions and reflections are summarized on the figure and were taken from the 60° line of Table II where the ratio of the intensities at the light-sensing apparatus is approximately 8 to 1.

Microscopic observations of the compound eye of *Daphnia pulex* reveal that the cone lens is not a circular solid cone of 45° surmounted by a hemisphere. Con-
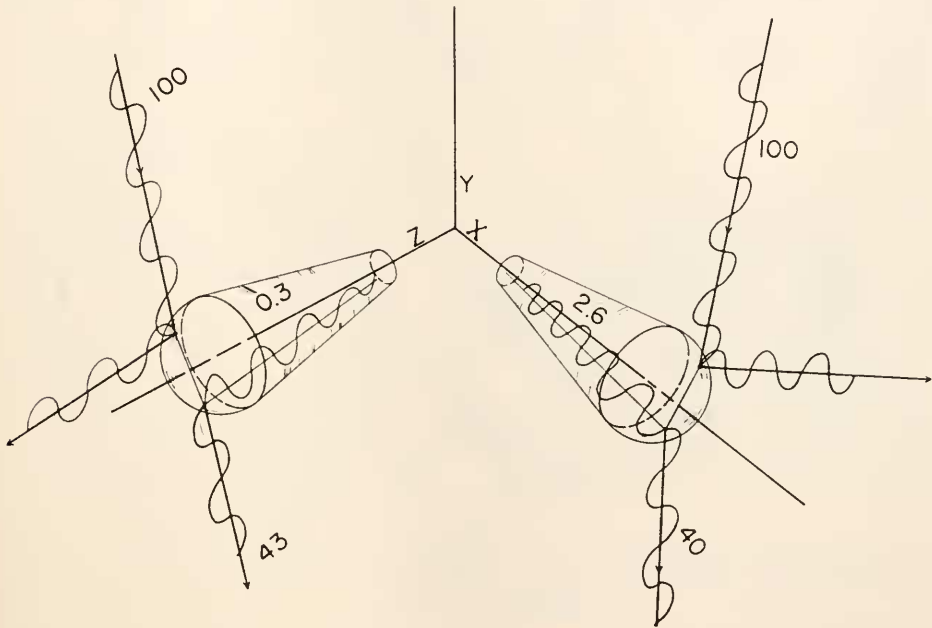

FIGURE 3. Three-dimensional diagram of the Brewster-Fresnel internal reflection model showing two cone lenses at right angles. Light rays are incident parallel to the y axis with an intensity of 100, and polarized parallel to the yz plane. The numbers represent calculated intensities at the various parts of the light path.

siderable variation in shape and contour is observed in the lenses of the eyes studied. In particular, one type of cone lens has a rather special shape in which the contours exhibited are of considerable theoretical interest because they are comparable to those predicted and drawn on paper from simple geometrical optical considerations. Starting with the knowledge that the light-sensing apparatus lies at the tip of the cone lens and with the constraint that the angle of the cone should be approximately 45° we may reconstruct the light path FDBA of Figure 1 through the cone lens step by step, starting at the apex and working backward to the outside
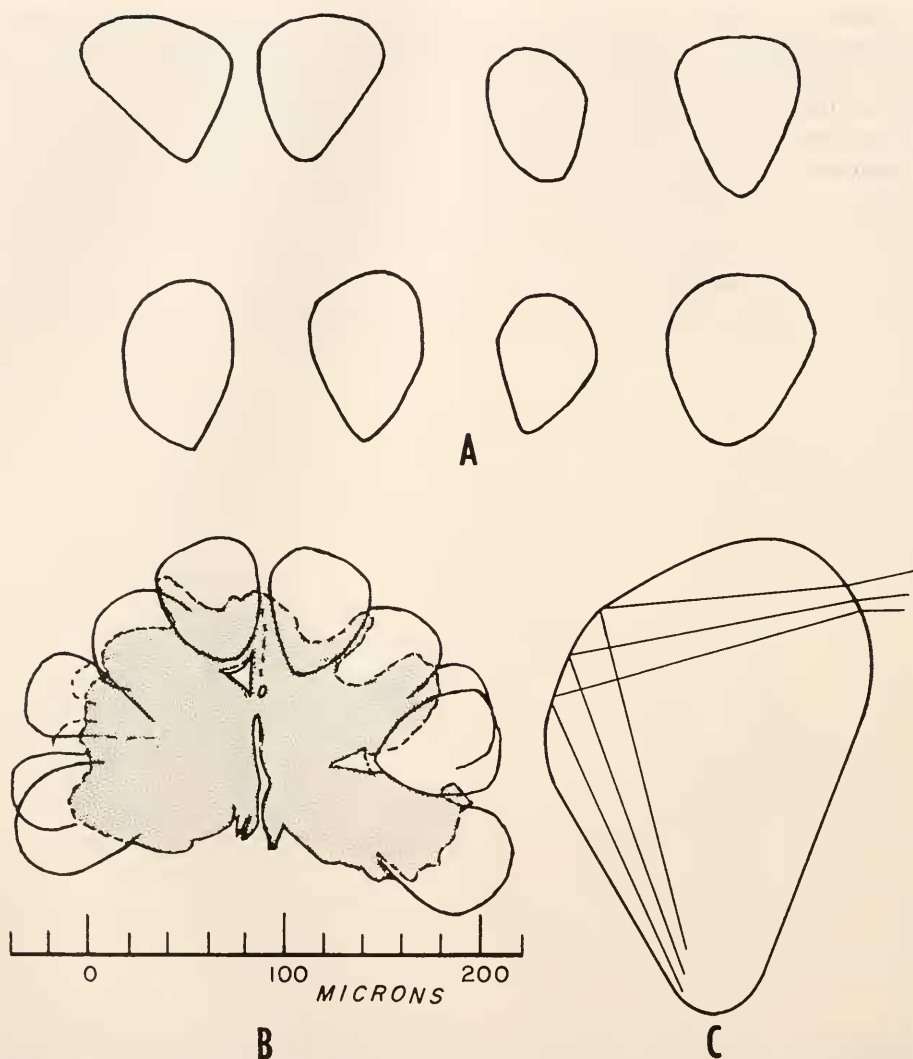
FIGURE 4. A, Outlines of selected cone lenses of *Daphnia pulex*. B, Composite tracing of photographs of three serial frontal sections through the compound eye of *Daphnia pulex*. Optic nerve protrudes from the center toward the 100-micron mark of the scale. C, Constructed lens with three light rays.

of the lens. A series of rays 5 to 10° apart are drawn from the apex toward the open end of the cone. For maximum efficiency of polarization detection each of these rays should be reflected from the periphery of the lens at the polarizing angle. Therefore, at the open end of the cone we construct across each ray a line which intersects the ray at this polarizing angle. The distances along the rays from the apex to the intersections are adjusted so that the constructed lines intersecting the rays produce a smooth curve. At each intersection of a ray with the curve

so produced a line is drawn perpendicular to it to permit construction of the reflected ray DB of Figure 1. The reflected rays are extended across the long axis of the cone toward B. A second intersecting surface is constructed across the rays to form a smooth curve which refracts all rays outward into a parallel bundle. The two smoothed theoretical surfaces are then joined across the base of the cone to complete the constructed figure. The completed figure (Fig. 4 C) cannot be superimposed on any photographs of cone lenses (Fig. 4 A and B) but provides a better approximation to the actual figure than does a hemisphere. These observations are consistent with the hypothesis that the dioptric contours of some cone lenses are specialized for polarizing angle reflection of light beams traveling at right angles to the long axis of the cone. The observations are also consistent with the intensity ratios measured in the light beam DE of Figure 1 and summarized in Table I, which are higher than anticipated from the calculations in Table II. The observed dioptric contours may also serve to decrease the intensity of ambient light incident parallel to the long axis of the cone. Figure 4 shows a constructed lens with outline drawings of selected lenses. Changes of lens shape as a result of fixation appear to be small when photographs of fixed material are compared with those of living material.

It is difficult to see how any of the models for light polarization plane detection operate effectively in a natural situation where the intensity of polarized light with a particular direction of propagation incident upon a receptor is masked by and confused with the intensity of light, whether polarized or non-polarized, from all other sources. When this happens the receptors must be able to distinguish intensity differences of a few per cent if orientation is to be precise. If we assume the model to be a perfect detector in the sense that the NS detector receives all light polarized in the NS plane and rejects all light polarized in the EW plane, it is still subject to confusion by ambient non-polarized light. Even with 100% polarized light the intensity ratios present for comparison in the Brewster-Fresnel external reflection model of Stephens et al. are not as great as 2:1, whereas the Brewster-Fresnel internal reflection model and the Autrum model have a maximum theoretical intensity ratio of infinity. The Brewster-Fresnel internal reflection model proposed for the daphnid cone lens appears especially vulnerable to the criticisms outlined above because such a small percentage of the incident polarized light is transmitted to the light-sensing apparatus. Therefore, it might be assumed that the reflected brightness pattern is the sole orienting stimulus. That it is not has been shown by Baylor and Smith (1953) and by Waterman (1960) who showed that orientation remained in spite of careful filtration of the water. In a separate experiment Smith and Baylor (1960) used a small half-wave plate umbrella to rotate the polarization plane of only the light directly incident on the daphnid without altering the reflection pattern. Here the daphnid oriented only to the polarized light plane incident from overhead unless the water was deliberately made turbid by addition of yeast. The function of polarized light responses in nature remains to be demonstrated and the possibility should not be ignored that many cases of polarized light responses may be only laboratory curiosities.

magna lenses were begun. Also, we wish to acknowledge a similar set of calculations by G. Schreuder-van Zanten and J. W. Kuiper in a manuscript sent to us by Prof. Kuiper.

SUMMARY

1. Three models suggested to account for the ability of arthropods to detect the plane of linear polarized light are characterized.

2. Measurements of polarized light refracted through the cone lens of *Daphnia pulex* are summarized.

3. These measurements are compared with calculated intensities derived from one of the three models.

4. The shape of the cone lens of *Daphnia* and the specialization of their contours for polarization analysis are suggested.

5. The operation of the various models in nature is criticized.

LITERATURE CITED

BAYLOR, E. R., AND F. E. SMITH, 1953. The orientation of *Cladocera* to polarized light. *Amer. Nat.*, **87**: 97–101.

BAYLOR, EDWARD R., AND FREDERICK E. SMITH, 1958. Extra-ocular polarization analysis in the honey bee. *Anat. Rec.*, **132**: 411.

VON FRISCH, K., 1950. Die Sonne als Kompass im Leben der Bienen. *Experientia*, **6**: 210–221.

SMITH, FREDERICK E., AND EDWARD R. BAYLOR, 1960. Bees, *Daphnia* and polarized light. *Ecology*, **41**: 360–363.

STEPHENS, GROVER C., MILTON FINGERMAN AND F. A. BROWN, JR., 1953. The orientation of *Drosophila* to plane polarized light. *Ann. Ent. Soc. of Amer.*, **46**: 75–83.

STOCKHAMMER, K., 1956. Zur Wahrenemung der Schwingsrichtung linear polarisierten Lichtes bei Insekten. *Zeitschr. vergl. Physiol.*, **38**: 30–83.

STOCKHAMMER, K., 1959. Die Orientierung nach der Schwingungsrichtung linear polarisierten Lichts und ihre sinnesphysiologischen Grundlagen. *Erg. der Biol.*, **21**: 23–56.

DE VRIES, HESSEL, AND JAN W. KUIPER, 1958. Optics of the insect eye. *Annals New York Acad. Sci.*, **74**: 196–203.

WATERMAN, T. H., 1954. Directional sensitivity of single ommatidia in the compound eye of *Limulus*. *Proc. Nat. Acad. Sci.*, **40**: 252–257.

WATERMAN, T. H., 1960. Interaction of polarized light and turbidity in the orientation of *Daphnia* and *Mysidium*. *Zeitschr. vergl. Physiol.*, **43**: 149–172.